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Fetal SCN tissue transplanted into the third ventricle of hamsters bearing complete SCN lesions restores the circadian locomotor rhythm with a period that depends exclusively on the genetically determined period of the tissue donor. If the host is only partially lesioned and thus retains rhythmicity with its own genetically determined period, an implant from an animal of a different geotype can induce a second rhythm with a period determined by the donor genotype. Both rhythms can be present simultaneously in the record of such a "temporal chimera," interacting only superficially (i.e., not at the level of the pacemaker). Our data support the interpretation that under such circumstances the graft is able to capture part of the locomotor output of the circadian system but does not make functional connections with the host SCN pacemaking system.

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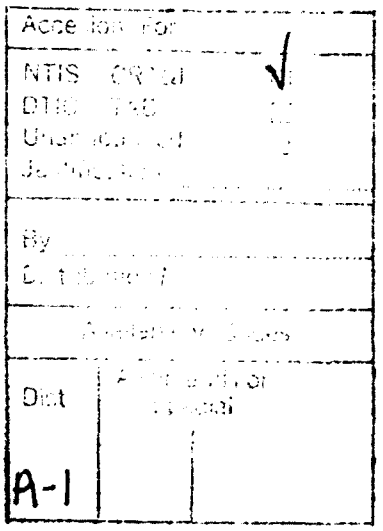
ABSTRACT

Fetal SCN tissue transplanted into the third ventricle of hamsters bearing complete SCN lesions restores the circadian locomotor rhythm with a period that depends exclusively on the genetically determined period of the tissue donor. If the host is only partially lesioned and thus retains rhythmicity with its own genetically determined period, an implant from an animal of a different genotype can induce a second rhythm with a period determined by the donor genotype. Both rhythms can be present simultaneously in the record of such a "temporal chimera," interacting only superficially (i.e., not at the level of the pacemaker). Our data support the interpretation that under such circumstances the graft is able to capture part of the locomotor output of the circadian system but does not make functional connections with the host SCN pacemaking system.



Transplantation of fetal SCN tissue into arrhythmic, SCN-lesioned host animals restores circadian locomotor rhythmcity (DeCoursey and Buggy 1989, Kawamura and Nihonmatsu 1985, Lehman *et al.* 1987). The circadian mutation *tau*, when present in homozygous form in hamsters, produces animals with free running periods of locomotor activity in constant darkness of *c.* 20 hours (Ralph and Menaker 1988). In SCN transplantation experiments, host animals that receive complete SCN lesions followed by a graft SCN from a fetal donor of a different *tau* genotype express circadian rhythms with period lengths that fall within the range of those expressed by intact animals of the donor genotype. In such experiments the proportion of animals in which circadian rhythmicity can be demonstrated subsequent to grafting varies between 40 and 90%; however, the dependence of the restored circadian period on the genotype of the donor is robust--indeed we have seen no exceptions (Ralph *et al.* 1990). Some of the data that support these statements are shown in Fig. 1; since their publication, many new data have been obtained which follow the same pattern. Thus the use of *tau* as a marker in SCN grafting experiments allows for the first time the unambiguous determination of the source of circadian rhythmicity in lesioned and/or grafted animals. That is important because several laboratories have reported that animals with SCN lesions (as judged by the occurrence of behavioral arrhythmicity following the lesion) have become rhythmic after several weeks or months of arrhythmicity even though they received no graft.

We have used the identifiability of the periods of rhythms restored by grafts to explore the behavioral consequences of supplying hamsters with two circadian oscillators with different genetically determined periods. We accomplished this by deliberately making partial SCN lesions in our host animals and implanting SCN tissue from animals of different *tau* genotype. We called animals produced in this way "temporal chimeras" because their behavioral rhythmicity contained separate elements with host and donor periods (Vogelbaum and Menaker 1992).

Our attempts to produce temporal chimeras were not always successful. Some animals showed only host periodicity (presumably because the graft did not "take"), while others displayed only donor periods (presumably because the initial SCN lesion left too little host tissue or the implantation procedure did further damage). However, we did obtain a large number (44) of animals that concurrently displayed both host and donor periods. In Fig. 2 (left hand panels) is shown a segment of data from a temporal chimera produced by partial lesion of a wild-type ($\tau \approx 24$ hr) host and implantation of an SCN from a homozygous *tau* donor ($\tau \approx 20$ hr). This same data segment has been plotted on 24, 22 and 20 hour timebases as indicated in the figure. Rhythms with periods of 20 and 24 hours are clearly present and 4 or 5 day bouts of activity with apparent periods around 22 hours can be seen in the data plotted on a 22 hour timebase. However, these "22 hour" components are probably artifactual, resulting from the interaction of the 20 and 24 hour rhythms (but not pacemakers). Indeed, we can model the data quite successfully by assuming that the locomotor output centers of the host animal sum inputs from two circadian oscillators, one of which has a period of 20 hours and the other a period of 24 hours, and each of which has an activity-suppressing as well as an activity-stimulating region (Vogelbaum and Menaker 1992).

In our model, which is shown in Fig. 3, locomotor activity is expressed when the sum of all inputs rises above a threshold. The right hand panels of Fig. 2 show specific model

outputs plotted on 24, 22 and 20 hour timebases. The excellent correspondence between the model output and the raw data suggest that: 1) there is no 22 hour oscillator in the chimeras and thus no demonstrable interaction between the host and donor oscillators at the pacemaker level; and 2) that the assumption of both a stimulatory and a suppressive output from both the host and donor SCN is well-founded. This is not the first (Davis and Menaker 1980) but is certainly the most compelling indication that the SCN has a circadian output that suppresses locomotor activity. Conceived of in this way, the mammalian circadian pacemaking system bears a remarkable resemblance to that of the cockroach (Page 1983, 1984) suggesting that broad organizational principles have either converged or been retained across great phylogenetic distances.

We have carried our analysis further by attempting to alter the relative amount of host and donor control over the expression of locomotor activity (Vogelbaum and Menaker submitted). We varied the size of the host lesion and the length of the time between lesion and implantation, as well as (inadvertently) the location of the implant in the third ventricle. Perhaps the most interesting finding from this series of experiments was that we were never able to see an effect of the SCN implant unless an SCN lesion had been made. The significance of this negative result is increased by the knowledge (derived from the experiments described above) that a graft can have an effect in the presence of a functional host SCN. In light of that fact, the requirement for a lesion to allow expression of the graft's rhythmicity suggests that, in some as yet unspecified way, the lesion allows the graft to gain access to the host's output system. This suggestion is reinforced by the results of experiments in which we varied lesion size: the larger the lesion, the higher the incidence of expression of donor rhythmicity.

In some experiments we implanted the donor tissue within minutes of making the lesion ("immediate" implants), while in others we implanted from 3 to 5 weeks after lesioning ("delayed" implants). There was no significant effect of the length of time between lesion and implantation on the expression of either donor or host rhythmicity. Upon histological inspection of the brains of the transplanted animals (3-4 months after the transplant procedure) we found that we could categorize the location of the implant as either 1) in the dorsal hypothalamus and/or mid-thalamic region, but not in the ventral part of the third ventricle, or 2) in the ventral part of the third ventricle, usually extending into the dorsal hypothalamus and/or mid-thalamic regions. The location of the implant did not affect the expression of donor rhythmicity, however there was a significant interaction between location and time of implantation. Many more delayed than immediate implants in location 1 expressed rhythmicity. This relationship did not hold for implants in location 2.

It is clear that the *tau* mutation is a useful marker of SCN function in studies involving SCN transplantation in golden hamsters. As a marker, it should prove valuable in implants of dissociated SCN cells (Silver *et al.* 1990), SCN tissue from donors of different ages and SCN tissue that has been treated in various ways before being implanted.

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FIGURE LEGENDS

Fig.1 Reciprocal transplantation of SCN tissue between wild-type and mutant animals. Periodicity was determined from eye-fit lines drawn through activity onsets on at least 20 consecutive days of data and was confirmed later with time series analysis. For each host, the endogenous rhythm (left) was eliminated by SCN ablation and restored by SCN implants (right). The range of period of the intact adult population for each genotype is indicated by vertical shaded bars (right axis). ***A.*** Reciprocal transplants between wild-type and homozygous mutants. ***B.*** Reciprocal transplants between wild-type and heterozygous mutants. Symbols represent the following: solid circle= host; open circle= SCN tissue from wild-type donor; *= SCN tissue from heterozygous donor; and open triangle= SCN tissue from homozygous mutant donor (from Ralph *et al.* 1990)

Fig. 2 A comparison of experimental data from a temporal chimera with the activity patterns predicted by simulations of the model shown in *Fig. 3*. ***Left panel:*** Wheel-running data of a wild-type hamster in constant darkness which received a partial SCN lesion followed immediately by a transplant of a hypothalamic block containing the SCN from a fetal homozygous mutant donor. Double-plotted data from days 55-84 following the lesion/implant are re-plotted on three timebases: 24, 22 and 20. ***Right panel:*** Activity patterns predicted by simulations of the model shown in *Fig. 3*. For the simulated data, the amplitude of each stimulatory and suppressive output was set equal to 50 and the threshold value was set equal to 20 (from Vogelbaum and Menaker 1992).

Fig. 3 Top A model of the control of expression of locomotor activity by host and transplanted circadian oscillators. Activity-stimulating (+) and activity-suppressive (-) inputs are received by the centers that drive locomotor activity. When the total level of input exceeds a threshold, these centers stimulate the expression of activity by the hamster; activity may also occur in response to stimuli not generated by the circadian system (not included in this model). ***Bottom*** The outputs of the host and donor circadian oscillators are modeled using the equation shown. Only the positive half of the sine wave function is used in the simulations of this model. The amplitude of each excitatory and inhibitory circadian output is determined independently. The stimulatory and suppressive outputs are maintained 180° out of phase for each of the simulations. The function *x(t)* describes the output from each oscillator shown at the *top*. Simulations run with this model are shown in *Fig. 2 (right panel)* (from Vogelbaum and Menaker 1992).

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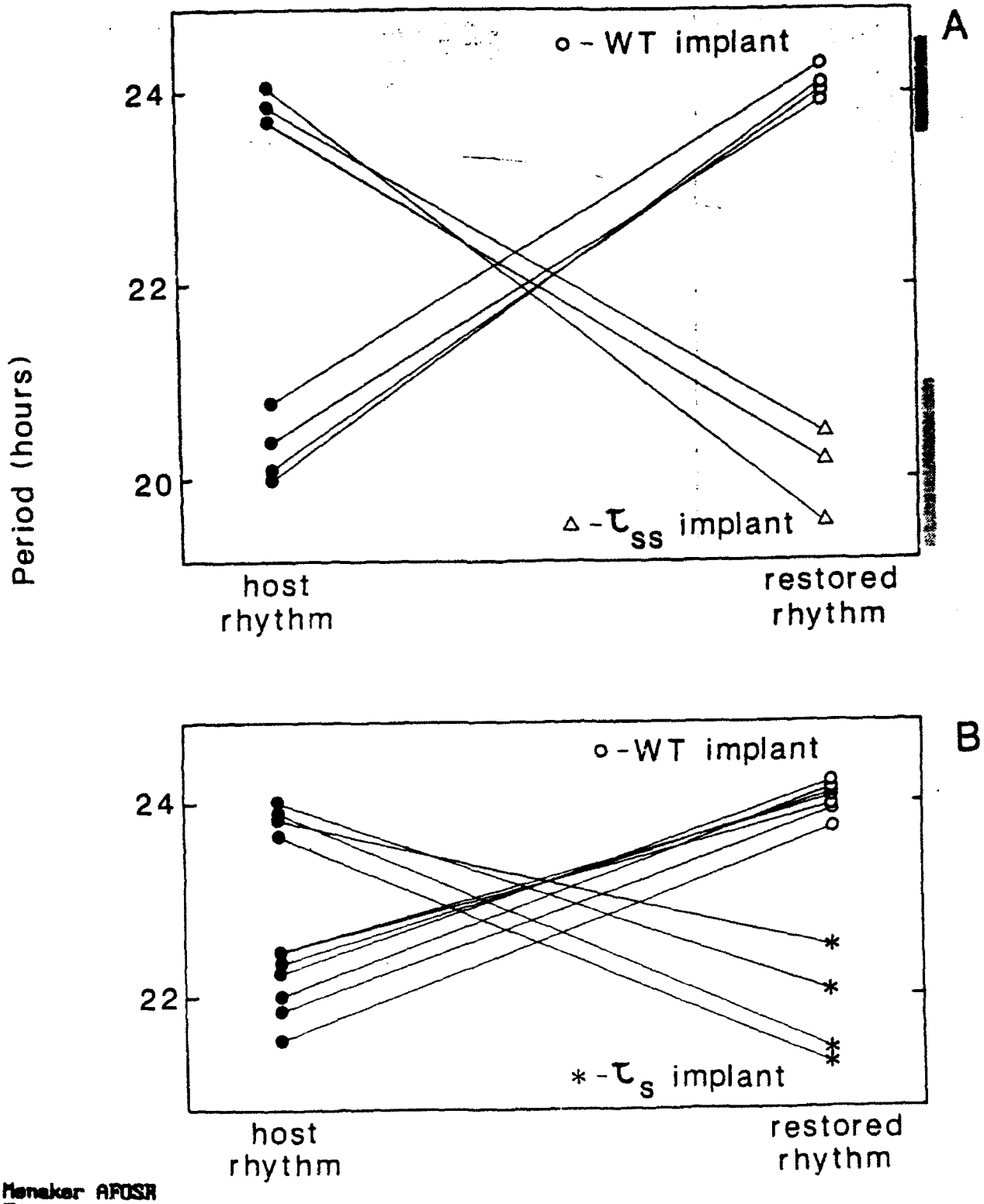

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Figure 1

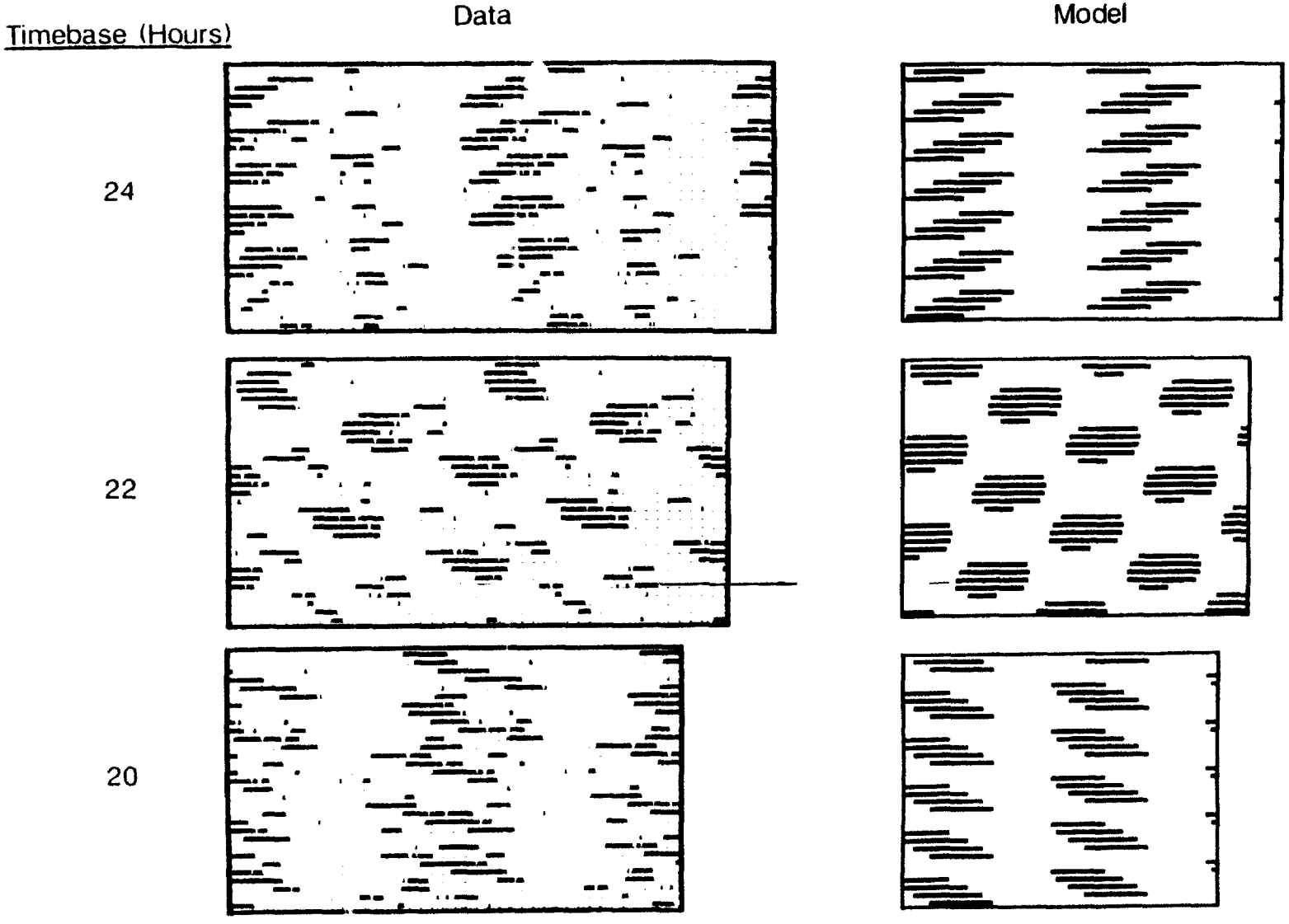

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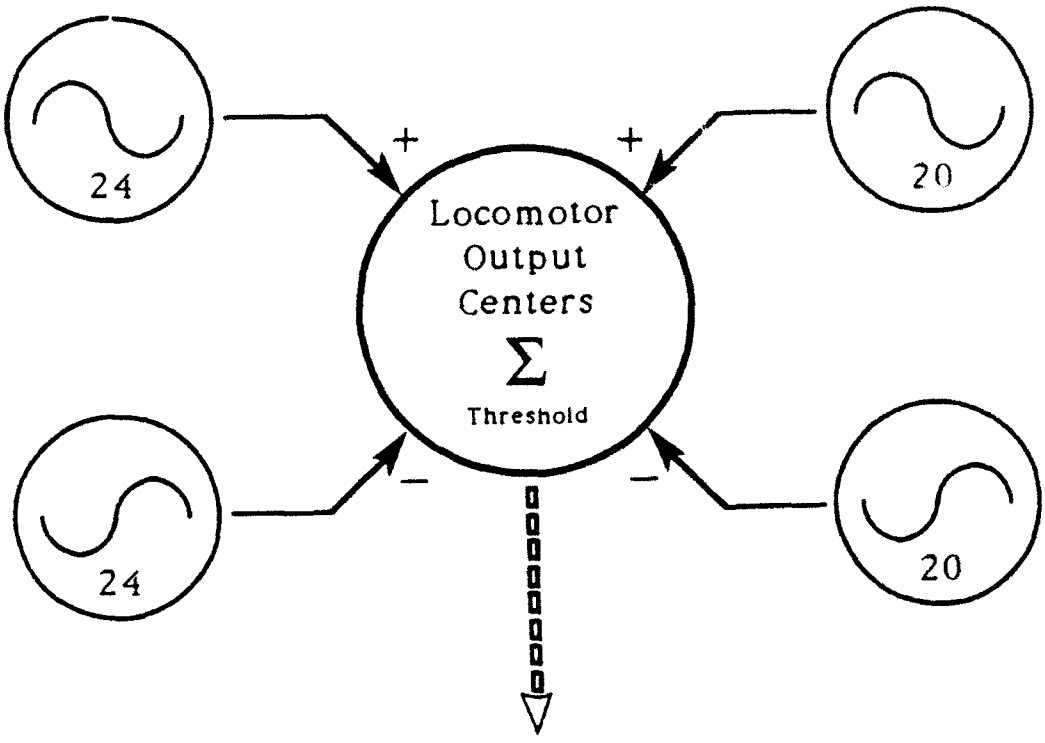
Figure 2



Model Equations

$$w(t) = A\sin((2\pi * t/\tau) - \phi)$$

$A \equiv$ amplitude

$\tau \equiv$ period

$\phi \equiv$ phase

For each rhythmic output.

$$x'(t) = \begin{cases} w(t) & w(t) \geq 0 \\ 0 & w(t) < 0 \end{cases}$$

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Figure 3